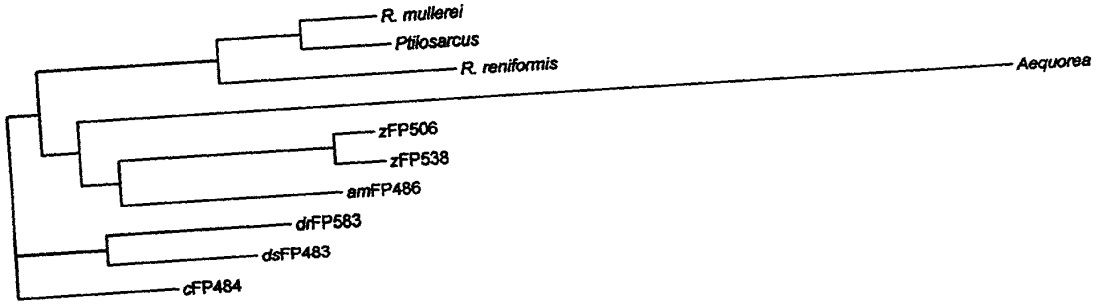

R. mullerei
Ptilosarcus
R. reniformis
Aequorea
zFP506
zFP538
amFP486
drFP583
dsFP483
cFP484

FIGURE 1

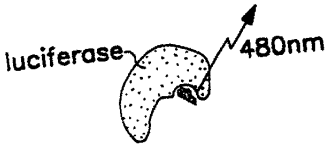


FIGURE 2A

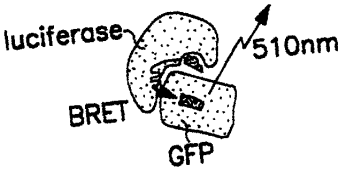


FIGURE 2C

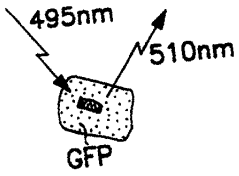


FIGURE 2B

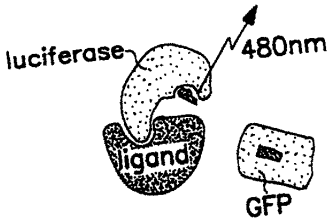
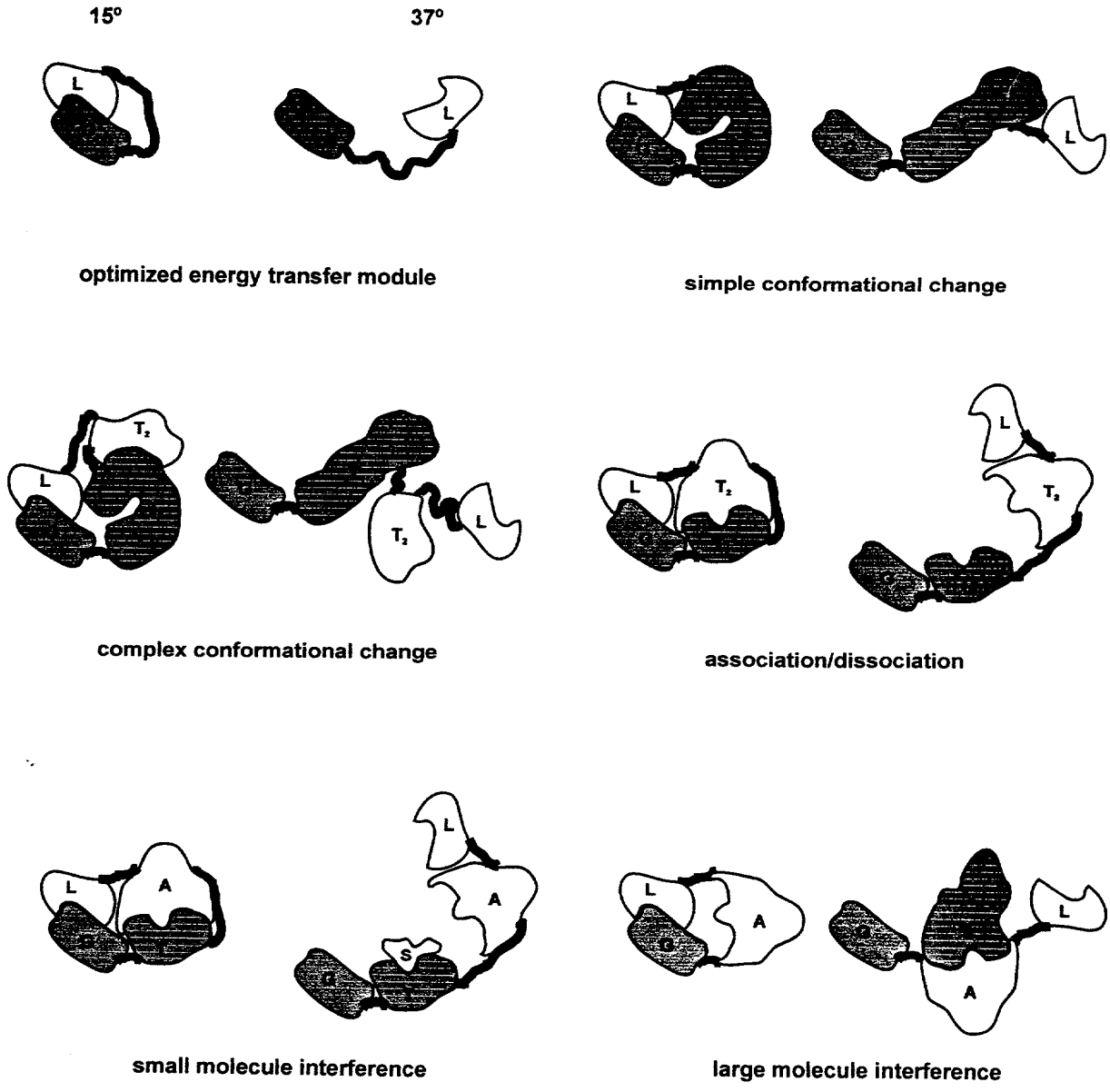


FIGURE 2D

FIGURE 2

BRET Sensor Architecture



BRET sensors are depicted for permissive and non-permissive binding states of the target molecules. Binding may be modulated by varying temperature or ionic strength.

FIGURE 3

Utilization of advantageous GFP surfaces with substituted fluorophores

```
                60          *          80
RM-GFP   :  GAPLPFAFDIVSPAFQYGNRTFTKYPNDIS--  :  83
Pt-GFP   :  GGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--  :  83
RR-GFP   :  GAPLPFAFDIVSVAFSYGNRAYTGYPEEIS--  :  80
cFP484   :  GAPLPFSYDILSNAFQYGNRALTKYPDDIA--  :  83
drFP583  :  GGPLPFAWDILSPQFQYGSKVYVKHPADIP--  :  80
asFP595  :  GGPLPFAFHILSTSCMYGSKTFIKYVSGIP--  :  77
dsFP483  :  GGPLPFGWHILCPQFQYGNKAFVHHPDNIH--  :  80
amFP486  :  GGPLAFSFDILSTVFKYGNRCFTAYPTSMP--  :  82
zFP506   :  GGPLPFAEDILSAAFNYGNRVFTEYPQDIV--  :  80
zFP538   :  GGPLPFSEDILSAGFKYGDRIFTEYPQDIV--  :  80
             ====================
```

FIGURE 4

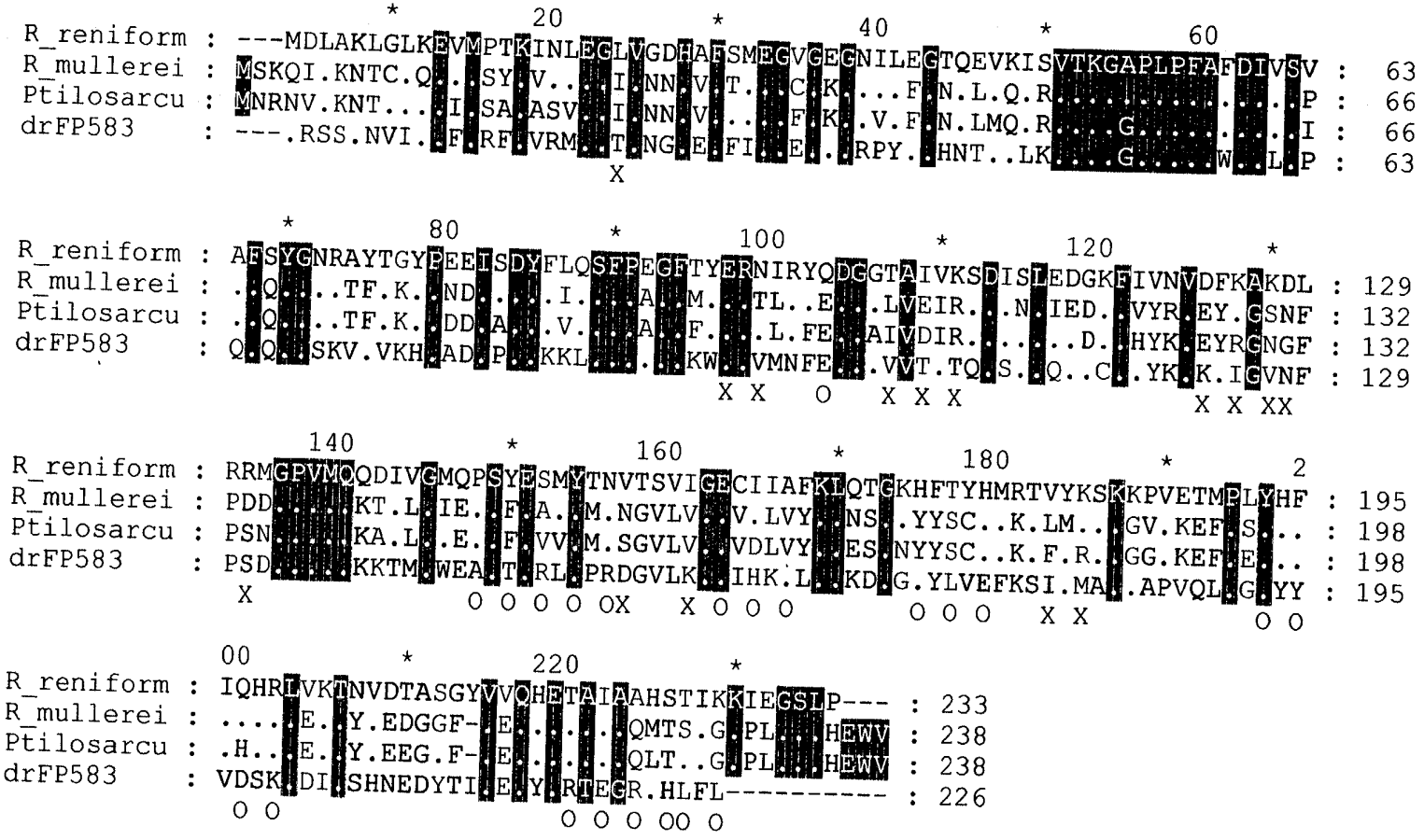


FIGURE 5

```
                    *         20        *         40        *         60        *         80
Aequorea    : ---MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTT---GKLPVPWPTLVTTFSYGVQCFSRYPDHMK :  79
R_mullerei  : MSKQILKNTCLQEVMSYKVNLEGIVNNHVFTMEGCGKGNILFGNQLVQIRVTK--GAPLPFAFDIVSPAFQYGNRTFTKYPNDI- :  82
Ptilosarcu  : MNRNVLKNTGLKEIMSAKASVEGIVNNHVFSMEGFGKGNVLFGNQLMQIRVTK--GGPLPFAFDIVSIAFQYGNRTFTKYPDDI- :  82
R_reniform  : ---MDLAKLGLKEVMPTKINLEGLVGDHAFSMEGVGEGNILEGTQEVKISVTK--GAPLPFAFDIVSVAFSYGNRAYTGYPEEI- :  79
drFP583     : ---MRSSKNVIKEFMRFKVRMEGTVNGHEFFIEGEGEGRPYEGHNTVKLKVTK--GGPLPFAWDILSPQFQYGSKVYVKHPADI- :  79
drFP593     : ---MSCSKNVIKEFMRFKVRMEGTVNGHEFEIKGEGEGRPYEGHCSVKLMVTK--GGPLPFAFDILSPQFQYGSKVYVKHPADI- :  79
dsFP483     : ---MSCSKSVIKEEMLIDLHLEGTFNGHYFFIKGKGKGQPNEGTNTVTLEVTK--GGPLPFGWHILCPQFQYGNKAFVHHPDNI- :  79
cFP484      : --KALTTMGVIKPDMKIKLKMEGNVNGHAFVIEGEGEGKPYDGTHTLNLEVKMAEGAPLPFSYDILSNAFQYGNRALTKYPDDI- :  82
asFP595     : ------MASFLKKTMPFKTTIEGTVNGHYFKCTGKGEGNPFEGTQEMKIEVIE--GGPLPFAFHILSTSCMYGSKTFIKYVSGI- :  76
amFP486     : ---MALSNKFIGDDMKMTYHMDGCVNGHYFTVKGEGNGKPYEGTQTSTFKVTMANGGPLAFSFDILSTVFKYGNRCFTAYPTSM- :  81
zFP538      : ---MAHSKHGLKEEMTMKYHMEGCVNGHKFVITGEGIGYPFKGKQ--TINLCVIEGGPLPFSEDILSAGFKYGDRIFTEYPQDI- :  79
zFP506      : ---MAQSKHGLTKEMTMKYRMEGCVDGHKFVITGEGIGYPFKGKQ--AINLCVVEGGPLPFAEDILSAAFNYGNRVFTEYPQDI- :  79

                    *       100       *       120       *       140       *       160       *
Aequorea    : RHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG--DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIK : 162
R_mullerei  : -SDYFIQSFPAGFMYERTLRYEDGGLVEIRSDINLIE--DKFVYRVEYKGSNFPDDGPVMQKTI-LGIEPSFEAMYM--NNGVLV : 161
Ptilosarcu  : -ADYFVQSFPAGFFYERNLRFEDGAIVDIRSDISLED--DKFHYKVEYRGNGFPSNGPVMQKAI-LGMEPSFEVVYM--NSGVLV : 161
R_reniform  : -SDYFLQSFPEGFTYERNIRYQDGGTAIVKSDISLED--GKFIVNVDFKAKDLRRMGPVMQQDI-VGMQPSYESMYT--NVTSVI : 158
drFP583     : -PDYKKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD--GCFIYKVKFIGVNFPSDGPVMQKKT-MGWEASTERLYP--RDGVLK : 158
drFP593     : -PDYKKLSFPEGFKWERVMNFEDGGVVTVSQDSSLKD--GCFIYEVKFIGVNFPSDGPVMQRRT-RGWEASSERLYP--RDGVLK : 158
dsFP483     : -HDYLKLSFPEGYTWERSMHFEDGGLCCITNDISLTG--NCFYYDIKFTGLNFPTNGPVVQKKT-TGWEPSTERLYP--RDGVLI : 158
cFP484      : -ADYFKQSFPEGYSWERTMTFEDKGIVKVKSDISMEE--DSFIYEIRFDGMNFPPNGPVMQKKT-LKWEPSTEIMYV--RDGVLV : 161
asFP595     : -PDYFKQSFPEGFTWERTTTYEDGGFLTAHQDTSLDG--DCLVYKVKILGNNFPADGPVMQNKA-GRWEPATEIVYE--VDGVLR : 155
amFP486     : -PDYFKQAFPDGMSYERTFTYEDGGVATASWEISLKG--NCFEHKSTFHGVNFPADGPVMAKKT-TGWDPSFEKMTV--CDGILK : 160
zFP538      : -VDYFKNSCPAGYTWGRSFLFEDGAVCICNVDITVSVKENCIYHKSIFNGMNFPADGPVMKKMT-TNWEASCEKIMPVPKQGILK : 162
zFP506      : -VDYFKNSCPAGYTWDRSFLFEDGAVCICNADITVSVEENCMYHESKFYGVNFPADGPVMKKMT-DNWEPSCEKIIPVPKQGILK : 162

                    180       *       200       *       220       *       240       *
Aequorea    : VNEKIRHNIEDGSVQLADHYQQNTPIG-DGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK---- : 238
R_mullerei  : GEVILVYKLNSGKYYSCHMKTLMKSKG--VVKEFPSYHFIQHRLEKTYVEDGGF--VEQHETAIAQMTSIGKPLGSLHEWV : 238
Ptilosarcu  : GEVDLVYKLESGNYYSCHMKTFYRSKG--GVKEFPEYHFIHHRLEKTYVEEGSF--VEQHETAIAQLTTIGKPLGSLHEWV : 238
R_reniform  : GECIIAFKLQTGKHFTYHMRTVYKSKK--PVETMPLYHFIQHRLVKTNVDTASG-YVVQHETAIAAHSTIKKIEGSLP--- : 233
drFP583     : GEIHKALKLKDGGHYLVEFKSIYMAKK--APVQLPGYYYVDSKLDITSHNEDYT-IVEQYERTEGRHHLFL---------- : 226
drFP593     : GDIHMALRLEGGGHYLVEFKSIYMVKK--PSVQLPGYYYVDSKLDMTSHNEDYT-VVEQYEKTQGRHHPFIKPLQ------ : 230
dsFP483     : GDIHHALTVEGGGHYACDIKTVYRAKK--AALKMPGYHYVDTKLVIWNNDKEFM-KVEEHEIAVARHHPFYEPKKDK---- : 232
cFP484      : GDISHSLLLEGGGHYRCDFKSIYKAK---KVVKLPDYHFVDHRIEILNHDKDYN-KVTLYENAVARYSLLPSQA------- : 231
asFP595     : GQSIMALKCPGGRHLTCHLHTTYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKLGHN--- : 232
amFP486     : GDVTAFLMLQGGGNYRCQFHTSYKTK---KPVTMPPNHVVEHRIARTDLDKGGN-SVQLTEHAVAHITSVVPF-------- : 229
zFP538      : GDVSMYLLLKDGGRYRCQFDTVYKAK--SVPSKMPEWHFIQHKLLREDRSDAKNQKWQLTEHAIAFPSALA---------- : 231
zFP506      : GDVSMYLLLKDGGRLRCQFDTVYKAK--SVPRKMPDWHFIQHKLTREDRSDAKNQKWHLTEHAIASGSALP---------- : 231
```

D,E,H,K,R	N,Q,S,T	L,I,V,M,F,Y,W	A,G	C,P
polar charged	polar uncharged	non-polar hydrophobic	small	not grouped

dimerization surfaces: ☐ hydrophilic ☐ hydrophobic

FIGURE 6